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Maternal serum oestrogen and androgen concentrations in preeclamptic and uncomplicated pregnancies

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Background Epidemiological studies show a substantially reduced risk of breast cancer in adult daughters of preeclamptic pregnancies, and modest risk reductions have been demonstrated for mothers also. Alterations in pregnancy hormone concentrations, particularly lower *in utero* exposure to oestrogen, are hypothesized to mediate this association.

Methods Pregnancy hormone concentrations were measured in maternal sera collected at hospital admission for labour and delivery from 86 preeclamptic and 86 uncomplicated, singleton pregnancies matched on length of gestation, maternal age, parity, and type of delivery.

Results Case and control pregnancies were similar in several maternal and pregnancy factors. Serum unconjugated oestradiol, oestrone, and oestriol concentrations were not lower in preeclamptic pregnancies in a matched analysis with adjustment for race and whether blood was collected before or after labour commenced. Serum unconjugated androstenedione (506.3 versus 316.0 ng/dl; $P = 0.0007$) and testosterone concentrations (214.5 versus 141.9 ng/dl; $P = 0.004$), however, were significantly higher in preeclamptic compared with control pregnancies, whereas dehydroepiandrosterone (DHEA) and DHEA sulphate did not differ.

Conclusions These data do not support the hypothesis that cancer risk in mothers and offspring of preeclamptic pregnancies is explained by exposure to lower maternal blood oestrogen concentrations, but raise the possibility that androgens play a role.

Keywords Hormones, preeclampsia, cancer, oestrogens, androgens, *in utero*

Interest in early life exposures and breast cancer has been fostered by data showing an elevated risk in daughters of pregnancies involving two placentas,[1–3] and a reduced risk in daughters of preeclamptic pregnancies.[1] Trichopoulos[4] hypothesized that elevations in concentrations of oestrogens and other hormones during pregnancy increase the probability of daughters developing breast cancer, and that this could explain the increased risk associated with a diplacental pregnancy, as well as the protective effect of preeclampsia, in mothers[5–7] and daughters, since virtually all studies had found lower urinary oestriol levels in women with this condition.[8–15]

However, subsequent studies measuring oestriol in blood from preeclamptic women have been inconsistent, with some studies showing lower oestriol values in preeclampsia[16–19] while others show either no difference[20,21] or higher concentrations.[22] Furthermore, few studies have assessed concentrations of other, more potent oestrogens such as oestradiol,[20,23,24] and oestrone.[23] Innes *et al.*[25] recently restated the oestrogen hypothesis and suggested that lower oestrogen concentrations in preeclampsia could be due to reduced aromatase activity which in turn could explain higher circulating androgen concentrations observed in some studies of preeclampsia.[18,23,24,26]

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In this study we attempt to characterize more completely the hormone profile of preeclamptic pregnancies by measuring androstenedione, testosterone, dehydroepiandrosterone (DHEA), and DHEA sulphate, in addition to oestriol, oestradiol, and oestrone, in maternal serum.

Patients and Methods

Study population

Subjects were a sample from an ongoing study of preeclamptic pregnancies being conducted at the Magee Womens Hospital, University of Pittsburgh. Of all deliveries at the hospital, 90% are among women from Pittsburgh, PA and Allegheny County, and comprise approximately 40% of all deliveries from these counties. The other 10% of deliveries at the hospital are from women who reside in West Virginia, Ohio and other counties in Western Pennsylvania. In the parent study, all women who delivered at Magee Womens Hospital (the entire obstetric service) from February 1994 through May 1998 and who were at least 14 years of age were invited to participate upon diagnosis of preeclampsia or at admission for labour and delivery. Overall participation by cases was approximately 80%. We included in our study the first 100 cases who agreed to participate after excluding those who had pre-gestational diabetes or hypertension, or who were pregnant with more than one fetus. All women attending the Magee Womens Hospital's obstetric practice (consisting of the hospital's clinic only) were invited to participate in the study as controls with the exceptions noted above for cases; 52% agreed to participate. In the present study, for every case, a control was chosen that matched the case as closely as possible on parity, length of pregnancy at delivery (± 2 weeks), type of delivery (vaginal versus C-section, and whether they laboured or not), and maternal age (± 5 years). When more than one control was identified as an eligible match, the control that most closely matched the case in the order of the matching variables stated above was chosen, and if there still remained more than one eligible control, the first on the list was chosen. Fourteen cases were excluded from the present study because either blood samples were not available during the appropriate time period, or the case could not be matched closely enough, leaving 86 preeclamptic cases and 86 uncomplicated controls for study. Informed consent for the questionnaire, interview, and blood collection was obtained from all study participants.

Case definition

Preeclampsia was defined by explicit criteria[27] and all diagnoses were reviewed by a jury of clinical experts. All three of the following criteria were satisfied with a documented return to normal values by 12 weeks postpartum. (1) Hypertension—compared with blood pressure averaged before 20 weeks gestation, an increase of 15 mmHg diastolic blood pressure (DBP) or 30 mmHg systolic blood pressure (SBP), or an increase in mean SBP of 20 mmHg using the last five blood pressure measurements in the hospital prior to anaesthesia or medication. With this approach 83% of women also had SBP >140 mmHg or DBP >90 mm Hg. If there were no blood pressure measurements available before 20 weeks gestation, then blood pressure >140 mmHg SBP or 90 mmHg DBP was considered hypertension (n = 7). (2) Proteinuria—a urinary dipstick protein value of 2+, or a urinary protein–creatinine ratio >0.3 on a random voided sample before rupture of membranes, or a value of 1+ or more on a catheterized urine specimen, or >500 mg/24 hours. (3) Hyperuricaemia—a serum uric acid value >1 SD above the mean for gestational age.

Blood collection and hormone assays

Sera were collected at hospital admission for labour and delivery from preeclamptic and normal pregnancies.

Samples were allowed to clot at room temperature, centrifuged, and stored at –80°C. Serum samples were analysed at Quest Diagnostics, San Juan Capistrano, CA. Laboratory personnel were blinded to case status, and case and control samples were run matched within batches to control for laboratory drift. Levels of unconjugated oestrone, oestradiol, and androstenedione were measured by an in-house method of radioimmunoassay (RIA) following extraction with organic solvent and purification by celite chromatography.[28,29] Unconjugated testosterone and oestriol were measured by extraction and RIA and DHEAs by dilution and RIA. Blinded aliquots of pooled sera from normal, pregnant women constituted 10% of each batch of study samples. The coefficients of variation for these blind replicates were 18.6% for DHEA, 8.5% for DHEA sulphate, 10.2% for androstendione, 9.6% for testosterone, 13.7% for oestradiol, 10.3% for oestrone, and 6.8% for oestriol.

Study variables

Information on demographics, reproductive and medical history, as well as some details of the pregnancy was obtained by interview and supplemented by subjects' medical records. Parity excludes the current pregnancy but gravidity does not.

Statistical analysis

All hormone values were logarithm-transformed because of skewed distributions. Means are presented on the natural scale for ease of interpretation (the antilogs of the logarithm-transformed values). Mean hormone concentrations were compared between preeclamptic and control pregnancies using analysis of covariance. Regression models accounted for the matched analysis by including a dummy variable representing each of the individual matches, which gives a *P*-value that is identical to that of a paired t-test and accounts for all interactions among the matching variables. Regression models included race (white/other), timing of blood collection (before/after labour started), and smoking (yes/no). Because the matching was not perfect, the distributions of the matching variables remained somewhat different between the cases and controls if there were no other covariates in the model. Therefore, regression models also included parity (nulliparous, 1, 2+), gravidity (0, 1, 2, 3, 4+), and weeks of gestation (continuous variable). The ratio of case to control means of hormone values is provided, based on the untransformed beta coefficient for case status from linear regression models with logarithm-transformed hormones as the dependent variable and the independent variables mentioned above. Comparisons of distributions of maternal, peri- and neonatal characteristics were tested using χ^2 statistics and t-tests. All analyses were performed using SAS software (Statistical Analysis System, Inc, Cary, NC).

Assuming an alpha of 0.05, 86 pairs and a SD = 6.5 (what we observed in the control group), the power was 99% to find a 20%

difference, 97% to find a 15% difference, and 72% to find a 10% difference in oestriol means between the cases and controls.

Results

Parity, pre-pregnancy weight, maternal height, smoking during pregnancy, delivery method, use and type of anaesthesia, and baby's sex were similar in cases and controls, while the cases were more likely to be white (Table 1). Cases were more likely to be in their first pregnancy compared with controls but this difference was not statistically significant. The babies of pre-eclamptic mothers had a slightly shorter gestation and after adjustment for gestational age, there were small differences in mean birth weight, birth length, and head circumference. Results were similar when the matching was considered in the analysis (data not shown).

Serum androstenedione and testosterone were significantly higher in preeclamptic compared with uncomplicated pregnancies (Table 2). Serum concentrations of oestradiol, oestrone, oestriol, DHEA, and DHEA sulphate did not differ significantly between preeclamptic and control pregnancies. Unadjusted and adjusted hormone analyses yielded the same pattern of results.

Blood was more likely to be drawn before labour started in preeclamptic pregnancies (74%) compared with controls (30%) since the prevalence of induction was greater among the former. Among women who had their blood drawn after labour started, length of labour before blood collection did not differ between cases and controls (5.9 hours and 5.6 hours, respectively; $P = 0.83$). In analyses restricted to controls, DHEA, DHEA sulphate, and oestrone were statistically significantly higher in women whose blood was collected after labour started (data not shown). Differences by timing of blood collection for the other hormones varied in direction and were not statistically significant. Timing of blood collection (before or after labour started) was treated as a possible confounding factor in all of the analyses comparing hormone concentrations between cases and controls. Mean time of day of blood collection differed significantly between cases and controls (11:00 and 14:30, respectively; $P < 0.0001$), but only DHEA showed a statistically significant variation with collection time when assessed by 4-hour intervals (data not shown). Adjusting for collection time in the final model for DHEA did not change the results (424 versus 418 ng/dl, respectively; $P = 0.91$). There were slight differences in the proportion of cases and controls that used the various types of

Table 1 Maternal, gestational, and perinatal characteristics in preeclamptic and uncomplicated pregnancies

Characteristic	Preeclampsia (N = 86)	Uncomplicated (N = 86)	*P*
Maternal age (y, $\bar{x}$ [SD])	26.5 (6.5)	26.2 (6.0)	0.90
Maternal race (%, [95% CI])			
White	70.9 (61–81)	58.1 (48–69)	
Black	25.6 (16–35)	39.5 (29–50)	
Other	3.5 (0–7.3)	2.3 (0–5.5)	0.06
Maternal gravidity[a] (%, [95% CI])			
1	54.7 (44–65)	40.7 (30–51)	
2	23.3 (14–32)	30.2 (21–40)	
3	12.8 (5.7–20)	15.1 (7.5–23)	
4+	9.3 (3.2–15)	14.0 (6.6–21)	0.32
Maternal parity[a] (%, [95% CI])			
0	74.4 (65–84)	73.3 (64–83)	
1	15.1 (7.5–23)	10.5 (4.0–17)	
2+	10.5 (4.0–17)	16.3 (8.5–24)	0.40
Pre-pregnancy weight (kg, $\bar{x}$ [SD])	66.3 (13.4)	69.1 (17.6)	0.34
Maternal height (cm, $\bar{x}$ [SD])	163.4 (7.3)	163.0 (5.9)	0.69
Smoked during pregnancy (%)	23.3 (14–32)	27.9 (18–37)	0.39
Weeks of gestation ($\bar{x}$ [SD])	37.0 (2.2)	37.8 (2.4)	0.04
Delivery method (%, [95% CI])			
Vaginal	80.2 (72–87)	81.4 (73–90)	
C-section	19.8 (11–28)	18.6 (10–27)	0.85
Anaesthesia (%, [95% CI])			
None	4.7 (0–9.1)	7.0 (1.6–12)	
Epidural	80.2 (72–87)	83.7 (76–92)	
Spinal	2.3 (0–5.5)	4.7 (0–9.1)	
Local	10.5 (4.0–17)	2.4 (0–5.5)	
General	2.3 (0–5.5)	1.2 (0–3.4)	0.21
Birthweight[b] (g, $\bar{x}$ [95% CI])	2704 (2502–2922)	2932 (2713–3168)	0.15
Birth length[b] (cm, $\bar{x}$ [95% CI])	48.2 (47.6–48.8)	49.1 (48.5–49.7)	0.05
Head circumference[b] (cm, $\bar{x}$ [95% CI])	32.9 (32.6–33.2)	33.7 (33.4–34.0)	0.0007
Male (%, [95%CI])	61.6 (51–72)	57.0 (47–67)	0.53

[a] Gravidity includes current pregnancy, parity does not.

[b] Means are geometric (exponentiated from logarithmic-transformed values) and adjusted for weeks of gestation.

Table 2 Maternal serum hormone concentrations in preeclamptic and uncomplicated pregnancies

Hormone	Preeclampsia (n = 86)	Uncomplicated (n = 86)
DHEA[a] (ng/dl)		
Median (range)	331 (71–1403)	432 (57–2478)
Adjusted[b] mean (95% CI)	446 (258–772)	430 (262–707)
Case/control ratio[c] (95% CI)	1.0 (0.8–1.4)	
DHEA sulphate (ug/dl)		
Median (range)	80 (8–278)	94 (11–461)
Adjusted[b] mean (95% CI)	58 (32–106)	69 (40–119)
Case/control ratio[c] (95% CI)	0.8 (0.6–1.2)	
Androstenedione (ng/dl)		
Median (range)	424 (79–2193)	356 (77–1719)
Adjusted[b] mean (95% CI)	444 (265–744)	276 (173–441)
Case/control ratio[c] (95% CI)	1.6 (1.2–2.1)	
Testosterone (ng/dl)		
Median (range)	185 (25–920)	150 (38–921)
Adjusted[b] mean (95% CI)	155 (90–267)	107 (65–176)
Case/control ratio[c] (95% CI)	1.5 (1.1–2.0)	
Oestradiol (pg/ml)		
Median (range)	24 047 (3880–71 216)	22 848 (815–75 137)
Adjusted[b] mean (95% CI)	20 800 (13 330–32 455)	18 370 (12 274–27 493)
Case/control ratio[c] (95% CI)	1.1 (0.9–1.3)	
Oestrone (pg/ml)		
Median (range)	6566 (680–30 813)	7682 (635–60 948)
Adjusted[b] mean (95% CI)	5272 (2844–9773)	5090 (2909–8905)
Case/control ratio[c] (95% CI)	1.0 (0.7–1.5)	
Oestriol (ng/ml)		
Median (range)	18 (2.3–37)	18 (1.1–35)
Adjusted mean (95% CI)	16 (11–23)	15 (11–21)
Case/control ratio[c] (95% CI)	1.1 (0.9–1.3)	

[a] Dehydroepiandrosterone.

[b] Adjusted means are geometric and are from models that included a variable representing the individual match, race (white/other), parity (nulliparous, 1, 2+), gravidity (1, 2, 3, 4+), smoking (yes/no), weeks of gestation, and timing of blood collected (before/after labour started).

[c] Case/control ratio is the exponentiated beta coefficient for case status from linear regression models with logarithm-transformed hormones as the dependent variable and the factors listed above as independent variables.

anaesthesia. The vast majority of women (80.2% in cases and 83.7% in controls) had an epidural but controlling for epidural versus other anaesthesia types in the final analyses did not appreciably change the results (data not shown). Women with preeclampsia were more likely to have been treated with magnesium sulphate (81.4% versus 1.2% (n = 1); $P < 0.001$) and prostaglandins (29.1% versus 8.1%; $P < 0.001$), and slightly more likely to be given oxytocin (84.9% versus 72.1%; $P = 0.06$) than controls. However, in over 60% of pregnancies, medications were administered after blood collection. The pattern of the results was similar excluding cases and controls administered medication before blood collection (data not shown).

Discussion

The reduced breast cancer risk in daughters, and perhaps mothers as well, associated with preeclamptic pregnancies is hypothesized to be mediated by exposure to lower oestrogen concentrations than would normally occur in an uncomplicated pregnancy.[4] Urinary oestriol concentrations are consistently reduced in women presenting with preeclampsia.[8–15] Our data however, provide no indication that maternal circulating levels of oestriol, oestrone, or oestradiol are lower in preeclamptic pregnancies. Instead, we found significantly increased levels of serum androgens, specifically androstendione and testosterone.

Differences in the ability to conjugate oestrogens may explain why concentrations in preeclampsia are consistently reduced in urine but not in circulating blood. In normal pregnancy roughly equal amounts of DHEA sulphate from the maternal and fetal adrenal glands are enzymatically converted in the placenta to androstenedione and testosterone, which are then aromatized to oestrone and oestradiol respectively, by the abundant aromatase enzyme.[30] Tenfold greater amounts of oestriol (200–300 mg/day at term) are also produced, derived from fetal adrenal DHEA sulphate which is first metabolized to 16a-hydroxy DHEA sulphate in the liver and then converted to oestriol in the placenta.[30] All of these oestrogens are secreted from the placenta into the maternal circulation principally in the unconjugated form, conjugated in the maternal liver and excreted in urine. Rosing *et al.*[23] speculated that the lower total circulating oestrogens they observed in preeclampsia despite similar concentrations of unconjugated serum oestrogen was consistent with reduced maternal hepatic blood flow, as a consequence of hypertension, decreasing the proportion of oestrogen conjugated in the liver.

Data on oestradiol concentrations in preeclampsia (a much more biologically active oestrogen than oestriol) are few. One large study showed significantly lower plasma oestradiol in

preeclampsia.[20] Of two smaller studies[23,24] that considered possible confounding by maternal and gestational factors, one[23] found lower *total* oestrogens in preeclampsia (which is mostly conjugated), and both,[23,24] like ours, found no difference in unconjugated oestrogen levels. Unconjugated oestrogen is arguably the more important parameter to measure since these levels are what enter the fetal circulation and are ultimately what the fetus is exposed to.

The significantly elevated serum androstenedione and testosterone concentrations in preeclamptic women are consistent with sparse, prior, relevant data.[24,26] Our findings of normal levels of DHEA, the substrate for androgen synthesis, in the face of elevated androstenedione and testosterone, are also consistent with two *in vitro* studies showing reduced conversion of androgens to oestrogens in placental tissue from preeclamptic pregnancies.

Siiteri (personal communication) has speculated on a possible role for androgens in decreasing breast cancer risk among mothers and daughters born of preeclamptic pregnancies. Low expression of the aromatase gene, or a small or impaired placenta, as found in preeclampsia, will increase the release of androgens from the placenta late in pregnancy when the fetal adrenal gland, the source of DHEA sulphate, undergoes rapid growth. Markedly elevated levels of these androgens result in virilization of female fetuses.[31] It is possible that less-dramatic elevations, accompanied by low sex-hormone binding globulin in fetal blood, might confer long-term protection against breast carcinogenesis by antagonizing oestrogen's effects on ductal development in the fetal breast.

Many of the studies of maternal hormone concentrations in preeclampsia have been limited by small numbers of preeclamptic women ($n < 40$),[12,16,18,23,24] lack of a well-defined comparison group,[13,14,16] or insufficient consideration of gestational age.[14,16,19,21] We attempted to minimize these limitations by designing a larger study with well-defined case and control groups and by controlling potential confounding. Nonetheless, there are several limitations of our study.

The data on which this epidemiological analysis was based were collected in a clinical study and the response rate in the controls was low. Cases arose from the entire obstetric service which included patients from both private and hospital practices, whereas the comparison group of uncomplicated pregnancies was drawn from the hospital's practice only. Our results could be biased if private and hospital-practice patients differed with respect to factors associated with hormone concentrations. For example, there were higher proportions of black mothers and smokers in the control group, and higher maternal testosterone levels in the early pregnancies of black women compared with white women have been observed,[32] as well as lower oestrogens in mothers who smoked during pregnancy.[33–35] Our results remained, however, with adjustment for race and smoking although we cannot discount confounding by other factors. The timing of blood collection (occurred after labour and delivery started versus occurred before labour and delivery started, or there was no labour) was adjusted for in all of the analyses because it was associated with both case status and a few of the hormones, so is unlikely to have confounded the results. Larger numbers would be necessary to meaningfully evaluate whether the association of preeclampsia and hormone levels varied depending on timing of blood draw.

In conclusion, we found no difference in concentrations of maternal unconjugated oestrogen between preeclamptic and normal pregnancies; however, androstenedione and testosterone concentrations were significantly higher in preeclamptic mothers. These data are not consistent with the hypothesis that reduced cancer risk in mothers and offspring of preeclamptic pregnancies is explained by exposure to lower oestrogen concentrations as reflected in maternal sera, but warrant more attention to the possible role of androgens in early life exposures and subsequent breast cancer. In addition, future studies should attempt to address more comprehensively the changes in pregnancy hormonal and other endogenous exposures in conditions that are associated with subsequent breast cancer risk in mothers and offspring.

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KEY MESSAGES

- Mothers and daughters of preeclamptic pregnancies have a lower breast cancer risk.
- The alteration in risk is hypothesized to be mediated by lower circulating oestrogen concentrations.
- Oestrogen concentrations in this study were not lower in mothers experiencing preeclamptic pregnancies compared with those who had uncomplicated pregnancies.
- These data do not support the proposed oestrogen hypothesis.

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